

Levels and health risk assessment of organochlorine pesticide residues in vegetables from Yamaltu area in Gombe, Nigeria

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The study determined the levels of organochlorine pesticides (OCPs) residues and assesses health risks linked with the consumption of vegetables cultivated in the Yamaltu area in Gombe, Nigeria. OCPs residues were solvent extracted and analyzed with a high performance liquid chromatography equipped with UV/VIS Detector. The mean concentrations of ten detected OCPs residues were almost all above the set limit of EU/WHOMRL. The estimated daily intake of OCPs from samples was below the acceptable daily intake, hazard index estimated were <1, indicated no probable non-carcinogenic health effect, while the carcinogenic health effect showed that children were more vulnerable for the consumption of the contaminated vegetables.

Introduction

Vegetables are an essential constituent of the human diet being sources of minerals salt and vitamins that are essential for the growth and boosting the immune system [1]. However, several studies revealed that fruits and leafy vegetables (eg. cabbage, lettuce, tomato, pepper, and onion) are vulnerable to metals and pesticide contamination [2-4]. In Nigeria and some other parts of the world researchers reported

significant contents of pesticide residues in different vegetables: carrot, spinach, garden egg, and cabbage [5], spinach and cabbage [6], onion [7], pepper, cameroon-green pepper, chili pepper, cabbage, carrot, lettuce and tomato [8], lady's finger, tomato and brinjal [9], carrot and cucumber [10], apples, grapes, pears, guava, and eggplants [11] and yellow peppers [12]. The contamination of these vegetables with residues of pesticide

derives primarily from the incessant use of these pesticides on vegetables [13], or poor agricultural practices especially in the developing countries [14].

Pesticides are mostly chemical substances that are frequently used in recent agriculture practices to prevent the crops from diverse diseases and pests [13, 15]. A great number of these pesticides have been used in agriculture [16]. They are also used to improve the agriculture yield considerably which leads to a dramatic increase in its application in recent years [17]. Farmers all around the globe use various kinds of pesticides which include carbamate, fungicides, pyrethroid, organochlorines, organophosphates, etc [9], to enhance agricultural yield and provide sufficient food supply for the growing world population [18]. The implication is that after application to a target place, a small amount of pesticides remain in or on crop after harvesting thereby making their way into the food chain [5]. Pesticide via oral ingestion path was found harmful compared to the other exposure [19]. Exposure of these toxic chemicals through oral ingestion could lead to severe and chronic health effects like: reproductive defects, disturbance of hormone, immune suppression, and even cancers at low exposure quantity [20, 21]. Regardless of the public health risks implication, an increase in the use of these pesticides in agriculture is still ongoing in Nigeria [22]. Currently, organophosphate and organochlorine pesticides

are widely used for agricultural and non-agricultural purposes in Nigeria even though the former has been banned in most countries due to their elevated persistence in the environment [5]. Organochlorines have received a lot of interest in recent times due to their inexpensive, persistency conformity against several pests, bio-accumulative character, and possible toxicity on wildlife and humans [7]. The organochlorine pesticides are recognized to induce changes in reproductive growth, behaviour or function in wildlife, and have been prohibited in developed countries but still in use in most Africa countries [23]. Organochlorine pesticides also reported to be associated with alteration of local environment and death of farm animals [24]. Even at low concentration, it's reported to act as sex hormones blockers, which leads to a sexual development changes, and also interfere with other hormonal processes which influences the development of bone [25].

In view of the information on the pesticides implications in the agricultural practices, it's therefore necessary to evaluate the risks linked with food exposure to pesticides to keep way harm to the consumers [26]. In the current study, the levels of organochlorine in four different vegetables from three different farms (Kwadan, Gadawo, and Kunji) cultivated in the Yamaltu area of Gombe, Nigeria was determined and health risks linked with vegetable consumption was also estimated.

Experimental part

Material and methods

Study area

The area of study is Yamaltu located about 9 km on Gombe-Biu road in southeast of the state capital Gombe, Nigeria. It lies between latitudes $10^{\circ}13' N$ and longitudes $11^{\circ}23' E$, 306 M above sea level. It is a rural area which covers a total land area of approximately 1,981 km². The study area is the Guinea savannah climate. The climate is tropical with two different seasons; a rainy and dry/harmattan seasons from (May-October) and (November-April) respectively with 30.5°C mean annual temperature and 62.5 mm precipitation, and with the relative humidity ranged from 58% to 72% annually [2]. **Figure 1** shows the sampling locations. The study area is a major vegetable producer (tomato, onion, pepper, chili pepper lettuce, cabbage, etc.) in the region.

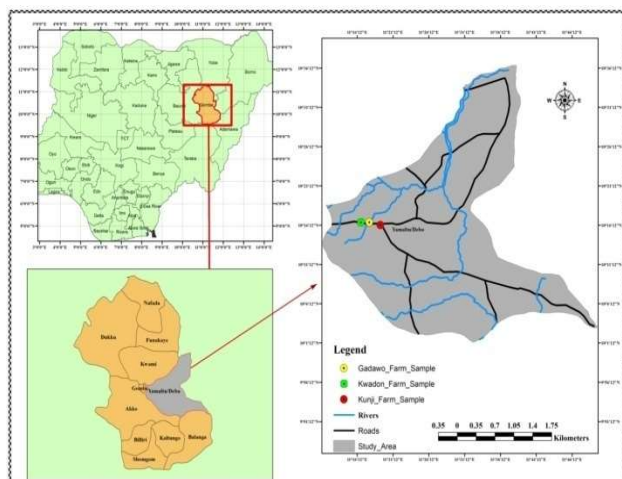


Figure 1. A map shows the sampling locations in Yamaltu, Gombe, Nigeria.

Pesticide standard and reagents

An analytical standard of pesticides, i.e mixed standard of organochlorine pesticides (OCPs)

with 99.9% purity; activated charcoal, acetonitrile (HPLC grade), anhydrous sodium sulphate, ethyl acetate and sodium chloride were purchased from Merck, Germany.

Samples collection

The samples of vegetables (tomato, onion, pepper, and chili pepper) were collected from three different farms (Kwadan, Gadawo, and Kunji) in the Yamaltu area. A total of 36 vegetable samples were collected for the study. The sampling sites are shown in **Figure 1**. The vegetable samples were obtained from diverse beds on each farm and bulked together to form composite samples. A total of 36 composite samples of tomato, onion, pepper, and chili pepper were collected for the OCP residue analysis. Each vegetable sample was placed in a poly bag and taken to the laboratory and stored in a refrigerator at 4°C before the analysis.

Samples extraction

Vegetables: 50 g of each homogenized vegetable was placed in a beaker and 50 ml of ethyl acetate/hexane/sodium chloride (3:1:1, v/v/v) was added and were then centrifuged for 5 min. The organic extracts were concentrated to 5 ml with a water bath at 45 °C using a vacuum rotary evaporator. The sample cleaning was done with a glass column having a 5 ml layer of anhydrous sodium sulphate and 10 g activated charcoal to eradicate any residual components that may possibly interfere with the high performance liquid chromatographic analysis, the extracts were evaporated to dryness and were further

redissolved in 5 ml of acetonitrile for the HPLC analysis.

Instrumental Analysis

The determination of the residues was carried out by injecting 1 µl of the 1.0 cm³ purified extract into the injection port of HPLC (Buck scientific (BLC10/11- model, Las Vegas USA), equipped with UV/VIS Detector set at 200-700 nm. The mobile phase with an initial composition consisting of 7% (v/v) solvent A (100% acetonitrile), and 93% of 20 mM KH₂PO₄ at a flow rate of 1 ml/min was maintained for seven min. Solvent A was then added initially 10% at 20 min, then increased to 15% at 25 min, then 20% at 30 min, and finally 25% at 45 min to 70 min. The programming was sustained in the isocratic mode in the following order: 40% A at 70.1 to 75.0 min and 7% A at 75.1 to 90.1 min. The column temperature was maintained at 30 °C, all detections were made at 338 nm.

Statistical data analysis

The data obtained from the analysis were statistically analyzed using SPSS version 20 for Windows. Analysis of variance was used to determine the variation among means at (P < 0.05) level of significance.

Quality assurance

The samples spiked with the concentration 0.01 mgL⁻¹ and 0.1 mgL⁻¹ standards of pesticides were prepared some hours prior to the extraction of pesticides in the samples and analyzed for the pesticides in order to check the analytical method for recovery, consistency, and efficiency.

The percentage mean recovery was evaluated using the expression below.

$$\text{Recovery percentage} = \frac{C_e}{C_m} \times 100 \quad \text{Eq. (1)}$$

Where C_m = the spiked concentration and C_e = experimental concentration.

The recoveries of spiked samples range from 95 to 110% demonstrating the reproducibility of the process used. The LOD ranged from 0.0011 to 0.021 and LOQ 0.0013 to 0.034 (µg kg⁻¹), the LOD and LOQ were determined by taking a signal to noise ratio of 3 times and 10 times.

Risk Assessment

The risk assessment was evaluated base on the recommended model by the United States Environmental Protection Agency's (USEPA) to estimate the health risk [7, 27].

Estimated Daily Intake (EDI)

The estimated daily intake was calculated according to USEPA guidelines to estimate health risks linked with the vegetable consumption with OCPs residues.

$$EDI = \frac{C_i \times F_r}{BW} \quad \text{Eq. (2)}$$

Where EDI = estimated daily intake, C_i = concentration of the pesticide residue (µg/kg), F_r = food rate consumption (kg·day⁻¹), the vegetable consumption rate was taken to be 0.137 µg/kg/day [7], and BW = average body weight (kg), the average body weight recommended by USEPA was used in the study (60 kg and 10 kg), for adults and children respectively [14, 28, 29].

Hazard Index (HI)

Hazard Index (HI) was evaluated as a fraction of estimated daily intake (EDI), and the acceptable daily intake (ADI). When HI is greater than one, it means that lifetime consumption of measured vegetables could be capable of causing health effects [30, 7].

$$HI = \frac{EDI}{ADI} \quad \text{Eq. (3)}$$

Where HI = Hazard Index for non-carcinogenic, EDI = estimated daily intake and ADI= acceptable daily intake, the ADI reported elsewhere was used in this study [8, 31].

Cancer risk

The carcinogenic risk linked with vegetable consumption was also estimated using the USEPA oral slope factor [32]. The Hazard Ratio (HR) was calculated using the equation 4. [33, 30, 34, 35, 7, 36].

$$HR = \frac{EDI}{BC} \quad \text{Eq. (4)}$$

Where HR = Hazard Ratio, BC = benchmark concentrations, the BC is derived by setting the risk to one in one million due to lifetime exposure [37, 7, 36]

$$BC = \frac{R \times BW}{Fr \times SF} \quad \text{Eq. (5)}$$

Where R = risk, F_r = food rate consumption and SF= slope factor

Results and discussion**Percentage and levels of OCPs residues in vegetable samples**

The percentage occurrence of OCPs is presented in **Figure 2**. The percentage level of aldrin in the samples was in the range of: 23.35, 22.60, 26.14 and 27.91 in tomato (TM), onion (ON), pepper (PP) and chili pepper (CP) respectively, while the percentage level of dieldrin in the vegetables were 11.66, 11.54, 17.29 and 59.51 in a similar order. The levels of δ -BHC in the vegetables were in the order of 24.54, 20.16, 25.72 and 29.58% in TM, ON, PP and CP, β -BHC has the following percentages in the same order: 14.75, 18.88, 24.25 and 24.10, while the γ -BHC distribution in the studied vegetables were 20.24%, 30.35%, 23.61% and 25.80% in TM, ON, PP, and CP respectively. The endosulfan I in the vegetables were in the order of 24.44, 24.67, 26.67 and 27.73 % in TM, ON, PP, and CP, while that of endosulfan II has the following percentages in the same order: 25.43, 23.03, 27.83, and 23.70 and endosulfan sulphate distribution in the studied vegetables were 26.18 %, 24.77 %, 24.53 % and 24.53% in TM, ON, PP, and CP respectively. The percentage levels of heptachlor in the vegetables were 22.73, 23.03, 26.00, and 28.23 respectively while that of heptachlor expoxide was 23.77, 25.32, 24.68, and 26.23 in similar orders.

Table 1. Levels of organochlorine pesticide (OCP) residues in vegetable (mg kg⁻¹) samples from Yamaltu area in Gombe, Nigeria

Organochlorines	Farms	Vegetable	Mean ±SD	Vegetable	Mean ±SD	Vegetable	Mean ±SD	Vegetable	Mean ±SD
Aldrin	Kwadan	Tomato	0.062±0.001	Onion	0.057±0.001	Pepper	0.060±0.001	Chili pepper	0.064±0.005
	Gadawo		0.044±0.002		0.045±0.001		0.062±0.001		0.065±0.001
	Kunji		0.052±0.001		0.051±0.000		0.055±0.001		0.060±0.001
δ-BHC	Kwadan	Tomato	0.060±0.001	Onion	0.052±0.001	Pepper	0.058±0.000	Chili pepper	0.066±0.001
	Gadawo		0.068±0.002		0.044±0.002		0.067±0.001		0.069±0.003
	Kunji		0.040±0.001		0.042±0.001		0.051±0.000		BDL
β- BHC	Kwadan	Tomato	BDL	Onion	BDL	Pepper	0.013±0.001	Chili pepper	0.014±0.001
	Gadawo		0.012±0.001		0.018±0.001		BDL		0.014±0.001
	Kunji		BDL		BDL		0.015±0.001		0.018±0.000
γ -BHC	Kwadan	Tomato	0.023±0.002	Onion	0.026±0.000	Pepper	BDL	Chili pepper	BDL
	Gadawo		0.032±0.001		0.032±0.001		0.043±0.001		0.148±0.122
	Kunji		0.032±0.001		0.028±0.001		BDL		BDL
Dieldrin	Kwadan	Tomato	0.053±0.001	Onion	BDL	Pepper	0.051±0.000	Chili pepper	0.056±0.001
	Gadawo		0.057±0.006		0.062±0.003		0.061±0.001		0.075±0.002
	Kunji		0.044±0.001		0.042±0.001		0.052±0.001		0.053±0.001
Endosulfan I	Kwadan	Tomato	0.034±0.001	Onion	0.029±0.001	Pepper	0.031±0.001	Chili pepper	0.026±0.000
	Gadawo		BDL		0.033±0.004		0.036±0.000		0.036±0.001
	Kunji		0.032±0.001		0.038±0.001		0.041±0.001		0.036±0.000
Endosulfan II	Kwadan	Tomato	0.027±0.001	Onion	0.024±0.001	Pepper	0.027±0.001	Chili pepper	0.022±0.000
	Gadawo		0.026±0.001		BDL		0.031±0.001		0.027±0.001
	Kunji		BDL		0.024±0.002		0.029±0.001		0.025±0.001
Endosulfan sulphate	Kwadan	Tomato	0.032±0.001	Onion	0.028±0.001	Pepper	0.034±0.001	Chili pepper	0.036±0.001
	Gadawo		0.030±0.000		0.033±0.003		0.032±0.001		0.026±0.001
	Kunji		0.033±0.001		0.029±0.001		0.025±0.001		0.027±0.001
Heptachlor	Kwadan	Tomato	0.015±0.002	Onion	0.015±0.001	Pepper	BDL	Chili pepper	0.018±0.000
	Gadawo		0.016±0.001		BDL		0.017±0.001		BDL
	Kunji		0.015±0.001		0.016±0.001		0.018±0.000		0.020±0.001
Heptachlor expoxide	Kwadan	Tomato	BDL	Onion	0.026±0.000	Pepper	0.025±0.001	Chili pepper	0.022±0.001
	Gadawo		0.026±0.001		0.028±0.001		0.023±0.001		0.032±0.001
	Kunji		0.026±0.000		0.029±0.002		0.033±0.001		0.032±0.001

BDL = below detectable limit, SD = standard deviation

Table 2. Health risk assessment of organochlorine pesticide residues in vegetable samples from Yamaltu area in Gombe, Nigeria

OCPs	BW (kg)	Tomatoes			Onions			Peppers			Chili peppers		
		EDI	HI	CR	EDI	HI	CR	EDI	HI	CR	EDI	HI	CR
Aldrin	Children	7.00 E-04	< 1	Yes	7.00 E-04	< 1	Yes	8.00 E-04	< 1	Yes	9.00 E-04	< 1	Yes
	Adults	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-04	< 1	No
δ -BHC	Children	8.00 E-04	< 1	NC	6.00 E-04	< 1	NC	8.00 E-04	< 1	NC	9.00 E-04	< 1	NC
	Adults	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	2.00 E-04	< 1	NC
β -BHC	Children	2.00 E-04	< 1	NC	2.00 E-04	< 1	NC	2.00 E-04	< 1	NC	2.00 E-04	< 1	NC
	Adults	0.00 E-00	0.0	NC	0.00 E-00	0.0	NC	0.00 E-00	0.0	NC	0.00 E-00	0.0	NC
γ -BHC	Children	4.00 E-04	< 1	NC	4.00 E-04	< 1	NC	6.00 E-04	< 1	NC	2.00 E-03	< 1	NC
	Adults	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	3.00 E-03	< 1	NC
Dieldrin	Children	7.00 E-04	< 1	Yes	7.00 E-04	< 1	Yes	7.00 E-04	< 1	Yes	8.00 E-04	< 1	Yes
	Adults	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-03	< 1	Yes
Endosulfan I	Children	5.00 E-04	< 1	NC	5.00 E-04	< 1	NC	5.00 E-04	< 1	NC	4.00 E-04	< 1	NC
	Adults	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC
Endosulfan II	Children	4.00 E-04	< 1	NC	3.00 E-04	< 1	NC	4.00 E-04	< 1	NC	3.00 E-04	< 1	NC
	Adults	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC
Endosulfan sulphate	Children	4.00 E-04	< 1	NC	4.00 E-04	< 1	NC	4.00 E-04	< 1	NC	4.00 E-04	< 1	NC
	Adults	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC	1.00 E-04	< 1	NC
Heptachlor	Children	2.00 E-04	< 1	Yes	2.00 E-04	< 1	Yes	2.00 E-04	< 1	Yes	3.00 E-04	< 1	Yes
	Adults	0.00 E-00	0.0	No	0.00 E-00	0.0	No	0.00 E-00	0.0	No	0.00E-00	0.0	No
Heptachlor expoxide	Children	4.00 E-04	< 1	Yes	4.00 E-04	< 1	Yes	7.00 E-04	< 1	Yes	4.00 E-04	< 1	Yes
	Adults	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-04	< 1	No	1.00 E-04	< 1	No

NC = not calculated, BW = body weight (10 kg for children and 60 kg for adults), EDI = estimated daily intake , HI = hazard index, CR = cancer risk

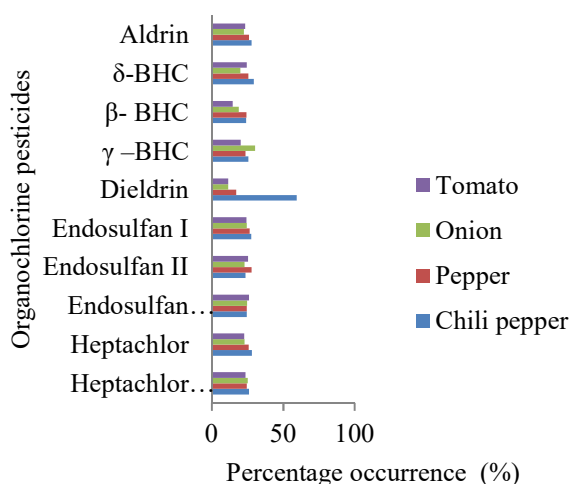


Figure 2. Percentage occurrence of OCPs in tomato, onion, pepper and chili pepper

The levels of OCPs in the vegetable samples are presented in **Table 1**. The mean concentration of aldrin in the vegetable samples ranged from 0.044-0.064 mg kg⁻¹ and the concentrations of dieldrin in the samples ranged from 0.00-0.075 mg kg⁻¹. δ-BHC concentration in the samples ranged from 0.00-0.069 mg kg⁻¹ and concentrations of β-BHC in the samples ranged from 0.00-0.014 mg kg⁻¹, while γ-BHC concentration ranged from 0.00-0.148 mg kg⁻¹ in the samples. The levels of endosulfan I in the samples ranged from 0.00-0.045 mg kg⁻¹, the endosulfan II ranged from 0.00-0.031 mg kg⁻¹ in the sampled vegetables, while that of endosulfan sulphate ranged from 0.025-0.036 mg kg⁻¹. The concentrations of heptachlor in the samples ranged from 0.00-0.020 mg kg⁻¹, while heptachlor expoxide concentration ranged from 0.00-0.033 mg kg⁻¹ in the samples. The level of aldrin obtained in this study was above (22.66 µg/kg) reported in vegetables from the farms of

Kumasi, Ghana [7]. Adeleye et al. [36] reported detection of aldrin and dieldrin in amaranths 0.509 and 0.205 mg kg⁻¹ and pumpkin 0.391 and 1.465 mg kg⁻¹ above the values obtained of this study. Ibrahim et al. also reported that the content of aldrin, dieldrin and endrin detected above the European Union maximum residue level (EU MRLs) [38]. The levels of benzene hexachloride (BHC) derivatives δ-BHC and γ-BHC except β-BHC were above the set limit of EUMRL [39]. However, δ-BHC was below the detectable limit in chili pepper from farm C, β-BHC in tomato from kwadan and Kunji, onion from kwadan, and gadawo, and pepper from gadawo while γ-BHC in pepper and chili pepper from kwadan and kunji respectively. Dieldrin was also below the detectable limit in onion from kwadan and recorded highest level 0.075 mg kg⁻¹ in chili pepper from gadawo and highest among detected OCPS. The high level of dieldrin in chili pepper might be due to its current usage, and also as aldrin breaks down to dieldrin [40]. The levels of dieldrin recorded in this study were higher than the value earlier reported in a similar study in foods grown in Nigeria [8]. Endosulfan I was below the detectable limit in onion from kunji, while endosulfan II was below the detectable limit in tomato and onion from kunji and gadawo respectively. The level of endosulfan sulphate detected in all the studied samples, was also above the set EUMRL. Chili pepper sample from kunji farm had the highest level of heptachlor and below the detectable limit

in onion, pepper, and chili pepper from gadawo, kwadan, and gadawo farms respectively, while heptachlor epoxide was below the detectable limit in tomato from kwadan and with the highest level in pepper from kunji farm. Moreover, this study shows that OCPs levels in vegetables were higher than those in early reported studies in other parts of the globe [41, 42, 43, 7, 8, 26]. This could be due to unawareness and misuse of the use of OCPs pesticides in Nigeria.

Health risk assessment

Table 2 presents the results of EDI, HI, and HR of OCPs via ingestion for children and adult. The hazard indices for the vegetable samples from the three studied farms were > 1 , for both children and adults. The estimate of the non-carcinogenic health risks obtained in study for children and adult were comparable with values obtained by Vincent et al. [7], but lower than the values reported in the previous studies. Adeleye et al. [36] reported that aldrin, dieldrin, endrin, endrin aldehyde, endosulfan sulfate and heptachlor posed non-carcinogenic health risk to children while aldrin, endrin, dieldrin, endrin aldehyde and heptachlor to adult. Adefemi et al. [44] reported that aldrin, endrin aldehyde, heptachlor and heptachlor epoxide posed non-carcinogenic health risk to children. Donkor et al. [45] also reported endrin aldehyde, heptachlor and heptachlor epoxide detected in tomatoes from Ghana pose health hazard to children. The carcinogenic risk showed that only dieldrin in chilli pepper posed carcinogenic health risks to

adult, while for children were dieldrin, aldrin, heptachlor epoxide and heptachlor in all vegetable samples. This result of this study is in line with previous study reported by Adeleye et al. that dieldrin and aldrin posed carcinogenic health risks to adult, while dieldrin, aldrin, heptachlor epoxide and heptachlor to children [36]. In contrast, report by Bolor et al. reported that carcinogenic health risk for vegetables from some selected farms in Ghana were < 1 [46]. The hazard ratio (HR) greater than one indicate carcinogenic health risk to its consumption [47, 48, 7, 36]. This suggests that children were more vulnerable to carcinogenic health risk for the consumption of the contaminated vegetables, considering the carcinogenic health risk were recorded in all vegetable samples. However, awareness and regular monitoring of OCPs and other pesticide residues are recommended, since an increase in misuse application of pesticides on agricultural produce is still ongoing in Nigeria.

Conclusion

The present study was conducted to determine levels of OCPs residue in vegetables and their associated health risks from the Yamaltu area in Gombe, Nigeria. Generally, the OCPs concentrations in the various vegetables were almost all above the permissible limit of EU/WHOMRL. The health risk assessment revealed that hazard index were less than one in samples for both children and adults, this an indication that no significant non-carcinogenic health risk considering the hazard index of the

OCPs were less than one, while the carcinogenic risk revealed that the studied OCPs posed carcinogenic effect to children, and dieldrin to adults. Hence, the need for regular monitoring of OCPs and other pesticide residues are recommended, since an increase in misuse application of pesticides to agricultural produce is still ongoing in Nigeria.

List of abbreviations

OCPs = Organochlorine Pesticides

HPLC = High Performance Liquid Chromatography

EU MRL = European Union Maximum Residue Level

LOD = Limit of Detection

LOQ = Limit of Quantitation

EDI = Estimated Daily Intake

BW = Body Weight

ADI = acceptable daily intake

HI = Hazard Index

CR = Cancer risk

HR = Hazard Ratio

BC = Benchmark Concentrations

BCH = Benzene Hexachloride

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